

Appl. No. : 10/063,699
Filed : May 8, 2002

AMENDMENTS TO THE SPECIFICATION

Please amend the title as follows:

~~SECRETED AND TRANSMEMBRANE POLYPEPTIDES AND NUCLEIC ACIDS~~
~~ENCODING THE SAME~~ A NUCLEIC ACID UNDER-EXPRESSED IN MELANOMA

Please amend the application to include the paper copy of the Sequence Listing submitted herewith.

Please amend paragraph 237, beginning at page 40, as follows:

--"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™ (a polyoxyethylene (20) sorbitan available from ICI Americas, Inc., Bridgewater, NJ), polyethylene glycol (PEG), and PLURONICS™ (a copolymer of propylene oxide and ethylene oxide available from BASF Corporation, Mount Olive, NJ).--

Please amend paragraph 337, beginning at page 93, as follows:

--The PRO polypeptides described herein may also be employed as therapeutic agents. The PRO polypeptides of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the PRO product hereof is combined in admixture with a pharmaceutically acceptable carrier vehicle. Therapeutic formulations are prepared for storage by mixing the active ingredient having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate,

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citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™ (a polyoxyethylene (20) sorbitan available from ICI Americas, Inc., Bridgewater, NJ), PLURONIC™ (a copolymer of propylene oxide and ethylene oxide available from BASF Corporation, Mount Olive, NJ) or PEG (polyethylene glycol).--

Please amend paragraph 405, beginning at page 111, as follows:

--Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate) (TAP Pharmaceuticals, Inc., Chicago, IL), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37° C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

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Please amend paragraph 435, beginning at page 117, as follows:

--When a positive colony was isolated, a portion of it was picked by a toothpick and diluted into sterile water (30 µl) in a 96 well plate. At this time, the positive colonies were either frozen and stored for subsequent analysis or immediately amplified. An aliquot of cells (5 µl) was used as a template for the PCR reaction in a 25 µl volume containing: 0.5 µl ~~Klentaq~~ KLENTAQ (a 5'-exo minus N-terminal deletion of Taq DNA polymerase available from Clontech, Palo Alto, CA); 4.0 µl 10 mM dNTP's (Perkin Elmer-Cetus); 2.5 µl ~~Klentaq~~ KLENTAQ buffer (Clontech); 0.25 µl forward oligo 1; 0.25 µl reverse oligo 2; 12.5 µl distilled water. The sequence of the forward oligonucleotide 1 was:--

Please amend paragraph 441, on page 119, as follows:

--Following the PCR, an aliquot of the reaction (5 µl) was examined by agarose gel electrophoresis in a 1% agarose gel using a Tris-Borate-EDTA (TBE) buffering system as described by Sambrook et al., supra. Clones resulting in a single strong PCR product larger than 400 bp were further analyzed by DNA sequencing after purification with a 96 ~~Qiaquick~~ QIAQUICK PCR clean-up column (Qiagen Inc., Chatsworth, CA)--

Please amend paragraph 446, on page 122-123, as follows:

--These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit and at least 5 years after the most recent request for the furnishing of a sample of the deposit was received by the depository. The deposits will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC § 122 and the Commissioner's rules pursuant thereto (including 37 CFR § 1.14. with particular reference to 886 OG 638).--

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Please amend paragraph 475, beginning on page 129 as follows:

--Twelve micrograms of the desired plasmid DNA is introduced into approximately 10 million CHO cells using commercially available transfection reagents ~~Superfect™ (Quiagen)~~ SUPERFECT™ (Qiagen), ~~desper™~~ DOSPER™ or ~~fugene™~~ FUGENETM (Boehringer Mannheim). The cells are grown as described in Lucas et al., supra. Approximately 3×10^7 cells are frozen in an ampule for further growth and production as described below.--

Please amend paragraph 489 beginning on page 131 as follows:

Recombinant baculovirus is generated by co-transfecting the above plasmid and ~~BaculoGold™~~ BACULOGOLD™ virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using ~~lipofectin~~ LIPOFECTIN cationic lipid (commercially available from GIBCO-BRL). After 4 - 5 days of incubation at 58°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression are performed as described by O'Reilley et al., Baculovirus expression vectors: A Laboratory Manual, Oxford: Oxford University Press (1994).--